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Induced pluripotent stem cells and its therapeutic potentials in regenerative medicine

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ABSTRACT

To avoid the complications of immune rejection of ES cells, diverse methods, such as somatic nuclear transfer (SCNT) and fusion of somatic cells with human ES cells, have been attempted to produce patient-specific pluripotent stem cells. Recent challenges are the process of generating induced pluripotent stem cells (iPSc) from adult somatic cells by certain defined factors through retroviral transfection method. These cells exhibited the properties of ES like cells in all aspects. Later, generation of Mouse iPS cells without viral vectors and generation of iPS cells without Myc are reported. iPSC cells offers a great therapeutic potentials in regenerative medicine by several findings, though therapeutic treatment of iPS is still at the earliest. However, continuous research on refining the novel therapeutic applications by circumventing the current problems is needed to make iPS cells a therapeutic potential for regenerative medicine.

KEYWORDS

iPS;
Pluripotent stem cells;
OCT4;
SOX2;
cMyc;
Klf4;
Nanog;
Lin28.

INTRODUCTION

Therapeutic use of stem cells depends on the availability of pluripotent cells that are not limited by technical, ethical, or immunological considerations. Recent work of somatic cell nuclear transfer (SCNT) technology opens the door to the possibility that SCNT of human cells will soon allow for the generation of "patient-specific" ES cells. An approach toward the same was recently described, were transduction of a set of four genes encoding the transcription factors Oct4, Sox2, C-Myc, and Klf4 globally reset the epigenetic and transcriptional state of fibroblast cells into that of pluripotent cells, designated induced pluripotent stem (iPS) cells, that were functionally indistinguishable from ES cells(7,14,15). Application of this approach in human cells would have enormous potential and generate patient-specific pluripotent stem cells to study and potentially ameliorate human disease.

Current prospects of induced pluripotent stem cells

Mouse iPSC's were first reported by Dr. Shinya Yamnaka his team at Kyoto University, Japan. They successfully reprogrammed mouse fibroblast in to pluripotent stem cells by transfection of four stem cell associated genes Oct 3/4, Sox 2, Klf 4 and C-Myc through

FULL PAPER

viral vectors, such as the retrovirus by antibiotic selection of FBX15$^+$. These cells they designated as iPSC's exhibit the morphology and growth properties of embryonic stem cells and express embryonic stem cell marker genes[14]. However, several differences were observed, for example: iPSC's showed DNA Methylation errors and failed to produce viable chimeras.

Professor Marius Wernig from Stanford University expanded upon epigenetic reprogramming of Yamanaka's cells by modifying the original protocol and uncovered that selection based upon FBX15$^+$ markers in Yamanaka's study isolated only "partially reprogrammed iPS cells", while Nanog and Oct 4 selection was able to identify " fully reprogrammed colonies"[18].

It was reported in November 2007 that human fibroblast can be transformed in to a pluripotent state that resembles that of Human embryonic stem cells. Takashaki and Yamanaka used same four transcription factors Oct 3/4, Sox 2, Klf 4 and C-Myc to reprogram human fibroblasts to iPSC's[15]. However, Reactivation of the c-Myc retrovirus, however, increases tumorigenicity in the chimeras and progeny mice, hindering clinical applications in humans. It was found later by different Scientists that a different set of four transgenes, OCT 4, SOX2, Nanog and Lin 28 can reprogram human somatic cells to iPS cells with similar efficiency (10-20 iPS cell colonies from 0.1 million initial fibroblasts)[3,4]. This suggests that C-Myc is not required for iPS generation and suggests that concerns with C-Myc reactivation causing tumors can be circumvented[8,17,23].

There has been an increasing research focus on the means of including the silencing of the retroviral inserted genes as the created cells might be prone to form tumors because of retrovirus. To overcome these dangers, Konrad Hochedlinger and his Harvard University research team used an adenovirus for transfection in to DNA of skin and liver cells of mice, resulting in cells identical to embryonic stem cells[9]. However adeno-iPS cells are not a perfect solution as the efficiency of generating adeno-iPS cells were much lower (0.001~0.0001%) than using conventional retroviruses (0.01~0.1%). The second drawback in adenoviral integration is it has not yet been tested on human cells. Yamanaka demonstrated that reprogramming can be accomplished via plasmid without any virus transfection system at all, although at very low efficiencies[12]. While this is a significant step towards generating iPS cells without side-effects, much work needs to be done to improve efficiency in creating them. Fortunately, other methods of generating integration-free iPS cells, such as chemicals, are promising[19].

Scholer's team in the year 2009 identified that OCT4 is the only driving force behind the conversion of neural stem cells in to iPS cells. Those cells which scholer's team calls "1FiPS" can differentiate in to all three germ layers giving rise to all body tissues and organs[6]. However the drawback remains in using OCT4 for creating iPS cells. For instance, two fold expression of OCT4 causes cell differentiation in to endoderm and mesoderm layers and down regulation of OCT4 results in differentiation of trophoectoderm[4]. Hence there are chances of high interference with signaling pathway involved in normal pluripotent stability.

Therapeutic potentials of induced pluripotent stem cells

Researchers cured sickle-cell anemia in a mouse model using iPS cells, highlighting the promise of iPS cells for future research. They reprogrammed mouse skin cells with retroviruses to produce the iPS cells, correcting the sickle-cell mutation in these cells using homologous recombination, differentiating these cells into blood-producing stem cells, and then transplanting the blood-producing stem cells into the mouse from which they were derived. After transplanting these cells into the mouse, it soon began producing healthy blood cells[5].

Scientists from Jaenisch lab investigators successfully reprogrammed immature Bcells in to iPS like cells with same four master regulator genes by transfection method. However, they found that an additional gene, C/EBP-alpha, was needed to nudge mature B cells to become IPS cells. The accomplishment highlights the power of the IPS cell approach and points toward mouse models that will aid in understanding autoimmune diseases such as multiple sclerosis and type 1 diabetes. Eventually, researchers will be able to study diseases by following a similar process with human cells, Jaenisch predicts.

The University of Wisconson Madison in 2009 found that generation of iPS cells from skin fibroblast taken from a spinal muscular atrophy expanded robustly

in culture maintained the disease genotype and generated motor neurons that showed selective deficits compared to those derived from the child's unaffected mother. This is the first study to show that human induced pluripotent stem cells can be used to model the specific pathology seen in a genetically inherited disease[3]. As such, it represents a promising resource to study disease mechanisms, screen new drug compounds and develop new therapies.

Later, Wernig discovered that iPS differentiated neuronal precursors can migrate and differentiate into neurons and glial cells after transplantation into mouse developing brains. Wernig used a GFP-expressing lentivirus to reprogram iPS cells that were later differentiated into neuronal precursor cells. Transplantation into lateral brain ventricles and analysis from GFP-expressions, he found high densities of cells in "septum, striatum, hypothalamus, and midbrain" tissues. Moreover, incorporated cells display various complex neuronal and glial morphologies, expressing the neuronal marker proteins NeuN and beta tubulin. Wernig then induced parkinson's disease like symptoms in rats by administering 6- hydroxyl dopamine to kill dopamine neurons and injected in the dorsal straitum of mice. To his surprise, 8 of the 9 mice tested showed stably recovery from the parkinsonian like symptoms only four weeks after transplantation[19].

Rudolf Janiesch and his team from the whitehead institute and Massacheusetts institute of technology collected fibroblast from patients with unexplained, idiopathic or sporadic Parkinson's disease and reprogrammed using a DOX -inducible lentiviral vector to transmit the pluripotency factors (13). Specifically, the factor gene sequences were placed within lox-P states that could be excised via cre-recombinase. After excision, it was observed that the factor-free iPS cells were more characteristically identical to ES cells. This technique is highly important because even the low activity of the virus inserted genes may alter potential for these human embryonic like cells, or induced pluripotent stem cells to differentiate in to other cell types or can cause cancer.

Later, Jenisch and his colleagues show that fibrblasts from the skin of five patients with idiopathic parkinsons disease can be efficiently reprogrammed and subsequently differentiated in to dopaminergic neurons using "Cre- recombinase excisable viruses" that could be inserted and then removed[19]. The major implications are that it offers a therapeutic potential for the generation of dopaminergic neurons that could be introduced into PD patients.

Future perspectives

Current application of iPS cells in regenerative medicine serves several hindrances. There remains the possibility that the very factors inducing the neuro degenerative disease in the human brain will lead to iPS apoptosis. Also, another disadvantage of using iPS reprogrammed cells in regenerative medicine is that the DNA microarray data has identified 1,267 genes that are expressed in vastly differing levels in iPS cells than in ES cells[4]. While the cascade mechanisms and functions of these genes remain unknown, the premature implantation of iPS cells into humans could lead to unexpected, harmful results. Still, the reprogrammed cells can be used to establish an effective in vitro model by which researchers can understand the pathophysiology of neurodegenerative disease. Such in vitro models could be utilized for large scale genetic or drug based screens since large number of hiPSC's can be generated and robustly differentiated in to dopaminergic neurons.

Hence, future research on refining of gene complements for efficient implantation of iPSC to humans needs to be done that will make iPS cell transplantation a safe procedure in humans. Another important step will be to identify ways of assessing which iPS cell lines are sufficiently reprogrammed and safe to use for therapeutic applications. Undoubtedly, this continued research on many novel therapeutic applications of iPS will bring about revolutionary solutions in the field of regenerative medicines in future.

CONCLUSION

Currently iPS cells can be used to create disease models to study the specific mechanisms and pathways involved, thereby modes of treatment is identified. The research on iPS cells will also allow the study of how drugs affect these individuals - called "Phase I Clinical trial in a dish". Nevertheless, advances in iPS research are occurring rapidly, therapeutic iPS treatment is still

FULL PAPER

at the earliest and several problems needs to be circumvented. However, iPS will bring revolutionary therapeutic changes in future years to come and serves a major solution in the fields of regenerative medicine.

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